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(54) Title: ASSAY SYSTEM**(57) Abstract**

A system (30) for assaying a fluid sample. The system (30) comprising a lens (33) capable of focussing both excitation and fluorescent radiation, a fluid-flow conducting conduit (42) being provided in the lens (33) extending transversely of the optical axis of and through the focal region of the latter. At least one mechanical screen (38A, 38B) is disposed adjacent the focal region in the conduit (42) to arrest passage of beads (40) as a function of bead diameter. The beads (40) are precoated with at least a moiety of a ligand/conjugated complex and preferably transparent to both the excitation and fluorescent radiation.

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ASSAY SYSTEM

1 This invention relates to chemical and biochemical assays, and more particularly to
2 an improved optical apparatus and methods for fluorescent assays.

3 Assays in which aliquots of sample-under-test and one or more reagents are
4 variously reacted in highly specific reactions to form ligand/conjugate complexes such as
5 antigen/antibody or similar complexes which may then be observed in order to assay the
6 sample for a titer of a predetermined moiety from the sample, are well known.
7 Typically, an antibody is used to assay for the presence of an antigen for which the
8 antibody is specific, but such assays have been extended to quantitate haptens such as
9 hormones, alkaloids, steroids, antigens, antibodies, nucleic acids, and fragments thereof,
10 and it is in this broad sense that the term "ligand/conjugate" as used herein should be
11 understood.

12 Sensitive immunoassays typically use tracer techniques in which a tagged
13 constituent of the complex is incorporated, for example in the reagent, the non-complexed
14 tagged reagent then being separated from the complexed reagent. The complexed can be
15 thereafter quantitated by observing a signal from the tag. Radioisotopes, fluorescent and
16 chemiluminescent molecules, colorimetric tags, and other markers have been used to label
17 constituents or moieties of the complex, appropriate apparatus being employed to detect
18 and measure the radiation from the label.

19 In such assays where at least one component of the conjugate complex is initially
20 bound to a solid substrate preparatory to formation of the complex, a basic problem arises
21 because of the typically lengthy time required to bind that component to the substrate.
22 For example, fluorescent assays such as those performed in the usual 96 well microtiter
23 plate, require time in the order of hours for binding of a component to the solid phase
24 to occur notwithstanding such expedients as heating, shaking and the like. It will be
25 appreciated that by increasing the surface area of the solid phase made available to
26 binding or coating with a ligand, the binding delay may be considerably reduced.
27 Consequently, the prior art relating to such solid phase assays (such as microtiter well
28 assays, dipstick assays and the like) also teaches using small particles or beads as the
29 solid phase.

30 Flowing the sample through a packed particulate bed speeds reactions between
31 the sample ligand being assayed and a conjugate immobilized on the surface of the
32 particles. Several factors probably contribute to this enhanced reactivity: the reduced
33 diffusion distance, the constant stirring of sample due to turbulent flow, and the high
34 density of binding sites in the reaction volume due to the high surface area exposed.

1 Known particle assays include the well-known bead agglutination test including
2 quantitative or semiquantitative slide agglutination and techniques in which the
3 agglutinated beads are separated from non-agglutinated beads by passage through a
4 mechanical filter. Another known particle assay is that described in U.S. Patent No.
5 4,780,423 in which particles with controlled porosity having ligand immobilized thereon
6 are incubated in suspension and washed. Washing can involve sedimentation and
7 resuspension of the particles. The resulting fluorescence can be read either from the
8 concentrated or the suspended particles. In yet another known assay, the particles are
9 bound to a membrane or filter through which the sample is then poured. This technique,
10 is believed to have been limited to enzyme-colorimetric detection. Where the particles
11 are incubated in a water suspension, the average diffusion distances which the free ligand
12 in the sample must traverse and the time required to bring complex formation to
13 completion tend to be quite large.

14 A principal object of the present invention is therefore to provide an improved
15 optical assay system in which the kinetics and sensitivity are improved by increasing the
16 surface area of the solid phase, decreasing diffusion distances, and enhancing the optical
17 coupling among the solid phase to the excitation light source and the coupling of the solid
18 phase to the detector. Another object of the present invention is to provide a novel flow
19 cell that provides the desired enhancement between the sample and a detector. Yet other
20 objects of the present invention are to provide such an assay system that requires small
21 sample volume and is particularly suitable for assay of whole blood; to provide such an
22 assay system in which the ligand/conjugate reaction is confined within a disposable item
23 that is readily insertable and removable from the optical system of the flow cell; and to
24 provide such an assay system in which all of the components of the desired complex other
25 than the sample moiety to be assayed, are preprovided.

26 Other objects of the present invention will in part be obvious and will in part appear
27 hereinafter. Generally, the foregoing and other objects of the present invention are
28 achieved by a system for assaying a fluid sample, typically employing a tag or label
29 intended to emit electromagnetic radiation when excited, the system comprising a flow
30 cell comprising hollow, light-transparent conduit means adapted for fluid flow
31 therethrough, and one or more separate porous masses of light-transparent material
32 disposed in the conduit means, the porosity of the mass of transparent material being
33 selected to permit fluid flow of the sample therethrough, at least a moiety of a respective
34 ligand/conjugate complex e.g. a specific-binding ligand, being immobilized, as by
35 precoating, on the surfaces of each mass.

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1 In one embodiment, the mass comprises a plurality of particles preferably
2 substantially transparent to light, particularly, where the complex formed includes a
3 fluorescent label, transparent to both radiation required to excite fluorescence and the
4 excited fluorescence. The particles are typically beads dimensioned within a specified
5 range of diameters and can be preformed, as by sintering or the like. Alternatively, the
6 mass can be formed by accretion against a fluid-porous barrier means disposed in the
7 conduit means. In the latter case, the barrier means is disposed within the conduit means
8 so as to define at least one wall of a chamber, the porosity of the barrier means being
9 sufficiently smaller than said range so that particles entrained in a fluid flow through the
10 conduit means are trapped by the barrier means and accrete to form the porous mass in
11 the chamber.

12 A preferred embodiment of the present invention includes focussing optical lens
13 mean through which the conduit means forms a hollow, tubular passage extending
14 transversely to the optical axis of and through the focal region of the lens means.
15 Typically, the lens means comprises a plurality of lenses and the conduit means extends
16 through one of those lenses. Where the system is to be used with a tag or label intended
17 to emit electromagnetic radiation when excited, the lens means must be capable of
18 focussing both the excitation and the emission radiation.

19 The invention accordingly comprises the apparatus possessing the construction,
20 combination of elements and arrangement of parts, and the method comprising the several
21 steps and the relation of one or more of such steps with respect to each of the others, all
22 as exemplified in the following detailed disclosure, and the scope of the application of
23 which will be indicated in the claims.

24 For a fuller understanding of the nature and objects of the present invention,
25 reference should be had to the following detailed description taken in connection with the
26 accompanying drawings in which like numerals in the several drawings are employed to
27 denote like parts, and wherein:

28 Fig. 1 is a diagrammatic representation, in cross-section, of assay apparatus
29 embodying the principles of the present invention;

30 Fig. 2 is a schematic cross-section of one embodiment of the flow cell of the
31 present invention;

32 Fig. 3 is a transverse cross-section of the flow cell of Fig. 2;

33 Fig. 4 is a schematic cross-section of a variation of the flow cell of Fig. 2;

34 Fig. 5 is a schematic cross-section of another variation of the flow cell of the
35 present invention;

1 Fig. 6 is a schematic cross-section of a variation of the flow cell of Fig. 5;
2 Fig. 7 is a schematic cross-section of another variation of the flow cell of Fig.
3 5; and

4 Fig. 8 is a schematic cross-section of yet another embodiment of the flow cell of
5 the present invention.

6 In Fig. 1 there is shown exemplary apparatus 20 for assaying a fluid sample and
7 which may typically employ an optical system including light source 22 for providing
8 excitation radiation, light detector 24 for detecting light stimulated by the excitation
9 radiation, beam splitter means such as dichroic or semitransparent mirror 26 and
10 collimator means 28. The embodiment of Figs. 1, 2 and 3 will be described, for ease
11 of exposition, for use particularly in the context of fluorescence immunoassay, but it
12 should be understood is not so limited. The term "light" as used herein will be
13 understood to include wavelengths in the visible spectrum as well as those in the near
14 infra-red and ultraviolet as well. Similarly, the term "excitation" will be understood to
15 include excitation of fluorescence, polarized or not, as by radiation, excitation of
16 chemiluminescence by chemical agents, emission by reflection of light from chromogens,
17 and the like.

18 The foregoing elements of the optical system are typically disposed in a frame
19 (not shown) in fixed optical relationship to one another, as described more fully
20 hereinafter. The invention further includes a flow cell 30, shown particularly in enlarged
21 form in Figs. 2 and 3, and in this embodiment, formed from a focussing optical lens
22 means 32 shown as a compound lens system including solid focussing lens 33, typically
23 made of glass, high molecular weight polymer or the like. Lens 33 is characterized by
24 having an elongated hollow channel or fluid-flow conducting conduit 34 therein directed
25 transversely to the optical axis of lens 32 and comprising a tubular passage, typically of
26 circular cross-section, through lens 33. At least a portion of such cylindrical conduit,
27 reaction chamber 36, is disposed at the focal region of lens means 32.

28 Thus, for example assume that fluid containing a ligand that can be excited, per
29 se or through an appropriate tag, into emission such as fluorescence, traverses chamber
30 34 and is appropriately excited into emission there by excitation radiation focussed onto
31 chamber 34 by lens means 32. That fluorescent emission is then directed by lens means
32 to detector 24 where, assuming that the detector for example is electrical, appropriate
33 electrical signals are produced and can be assessed to evaluate the fluorescence.

34 In order to provide a better signal-to-ligand ratio, the embodiment shown in Fig.
35 4 includes mechanical, fluid-porous barrier or screen 38 dimensioned and disposed in

1 conduit 34 adjacent the focal region of lens means 32 so as to arrest transport of particles
2 or beads 40 of predetermined size in a flow stream through the conduit. Such beads are
3 substantially transparent to both the excitation radiation and the excited fluorescence, and
4 to that end are typically formed of polymethylmethacrylate, styrene-divinylbenzene
5 copolymer or the like. Beads 40 are coated with at least a moiety of the antibody/antigen
6 complex, e.g. a specific-binding ligand, for example an antigen and an antibody thereto,
7 disposed at least on a portion of the surface of the bead.

8 The mesh or porosity of screen 38 is selected to allow free flow of sample fluid
9 and its constituents therethrough while arresting flow of the coated beads, and thereby
10 accreting a mass of beads 40 against the screen and in the focal region of the lens means
11 32. The particle size of the beads is selected to be minimized, provided however that
12 when a mass of beads is accreted against screen 38, the sample constituents may still pass
13 freely through the accretion mass. Typically, a bead size that works well with whole
14 blood as a sample is in the range of 50 μm to 250 μm , preferably around 98 μm . Bead
15 size, of course, depends to some extent on the nature of the sample (e.g. blood, food,
16 urine, process stream and the like). Mesh size, of course, depends upon the range of
17 diameters of the beads to be employed in the system, but typically, for beads of about 98
18 μm diameter, a mesh size of about 50 μm is appropriate. Thus, as sample fluid is flowed
19 through conduit 34, it must pass through the interstices of the accreted mass of coated
20 beads 40, resulting in a very small diffusion distance over which the assayed moiety must
21 pass to complex with the coating on the beads. This small diffusion distance, coupled
22 with the long, tortuous path of the sample through the accreted mass and the high surface
23 to volume ratio of the beads, enables very efficient scavenging of the assayed moiety
24 from the sample. This characteristic of the present invention is significant inasmuch as
25 the diffusion time is reduced by the square of the diffusion distance. It should also be
26 noted that the entire solid phase is contained in the accreted mass, a very small volume
27 (e.g. about 0.02cm³ for a typical conduit of 0.18 cm diameter), and is "immersed" in lens
28 32 thus providing a high numerical aperture, optical coupling between the excitation and
29 detection systems. Because the fluorescent signal is increased by the fourth power of the
30 numerical aperture, high numerical aperture optical coupling is very important.

31 In operation of the invention shown in Fig. 4, a quantity of beads 40 are preferably
32 preloaded with an appropriate ligand immobilized onto the bead surfaces by adsorption
33 or other known immobilizing techniques and suspended in a suspending fluid. Where the
34 beads will ordinarily not readily form a stable suspension in the suspending fluid, they
35 may be placed into a vortexer (not shown) or similar mixer which maintains the beads

1 in a suspension, typically aqueous, by agitation. A desired portion of the bead suspension
2 is sucked out of the vortexer as by a pump (not shown) and injected into conduit 34
3 where the flow of the beads is arrested by screen 38, creating an accretion or mass of
4 beads 40 within reaction chamber 36. An aliquot of sample solution being assayed is
5 then flowed through conduit 34 and the mass of beads 40 in reaction chamber 36,
6 effecting the formation of a ligand/conjugate complex on the surface of the beads. As
7 is well known, for competitive assays, prior to flowing the sample solution through the
8 flow cell, typically the sample solution is first treated with a tagging reagent and allowed
9 to incubate. Where the assay is a sandwich assay, the sample solution is passed through
10 the flow cell, then tagged antibody is passed through the cell, and the bead mass is
11 subjected to a wash step. As is well known in the art, a tagged, typically fluorescent,
12 component may be either the complement or conjugate to or an analog of the immobilized
13 ligand, depending upon whether a competitive or sandwich assay is to be performed. The
14 tag or label is typically a fluorescent dye such as a fluorescein dye, acridine dye or the
15 like, all as well known in the art. In either case, the resulting ligand/conjugate complex
16 should include desired dye moieties bound to the complex. Flowing a wash buffer
17 through the bead mass then washes out any unreacted materials and particularly any free
18 dye components, leaving only those dyed moieties as are immobilized on the beads.
19 Light source 22 is then activated to generate excitation light beam 23 (shown in broken
20 lines) which, in turn, directed to mirror 26 by collimating lens 28 so that the collimated
21 beam is reflected onto lens means 32. The latter focusses the excitation beam to a focal
22 region at which the mass of beads 40 in reaction chamber 36 is located, and the excitation
23 radiation excites the fluorophores on beads 40 into fluorescence. That fluorescence is
24 transmitted through lens 32 and directed through beam splitter mirror 26 to detector 24.
25 After measurements are made, the mass of beads 40 can be readily removed from
26 reaction chamber 36 simply by back-flushing through conduit 34.

27 As thus described, the technique of filling the reaction chamber from a suspension
28 or pool of preloaded beads is clearly amenable to automation, where the components for
29 specific assays, such as the type of preloaded beads, sample solution, tagging reagent and
30 the like, are selectable by appropriate valves controlling the flow of materials from
31 respective storage containers. However, the present invention also is readily adaptable
32 for more portable systems in which the bead mass and reagents are disposables.

33 For example, while conduit 34 is shown in Fig. 3 to be simply a passageway
34 through the focal region of lens means 32 transverse to the optical axis of the lens, in the
35 embodiment shown in Fig. 5, conduit 34 is formed of elongated bore 34A of uniform

1 diameter provided similarly through lens 33 and elongated light-transparent tube 42
2 having a uniform diameter slightly less than that of bore 34A so that tube 42 may be
3 inserted and removed from the bore. Screen 38 is so disposed within tube 42 that the
4 latter can be positioned within bore 34A adjacent the focal region of the lens.

5 In yet another embodiment of the flow cell of the present invention, as shown in
6 Fig. 6, conduit 34 is similarly formed of elongated bore 34A of uniform diameter through
7 lens 33 and elongated light-transparent tube 42A having a uniform diameter slightly less
8 than that of bore 34A so that tube 42 may be inserted and removed from the bore.
9 Screens 38A and 38B are so disposed within tube 42 in spaced-apart relation to one
10 another so as to define reaction chamber 44 within the tube. As in the embodiment of
11 Fig. 5, reaction chamber 44 can be positioned within bore 34A adjacent the focal region
12 of the lens. Included within chamber 44 is a plurality of beads 40 dimensioned within
13 a specified range of diameters, the mesh of screens being sufficiently smaller than the
14 range of bead diameters so that the latter are trapped by the screens in chamber 44 to
15 form a porous mass positionable substantially at the lens focal region. The beads in the
16 embodiment of Fig. 6 are preferably precoated with the desired specific binding ligand
17 before installation in chamber 44.

18 In both the embodiments of Figs. 5 and 6, it will be appreciated that tubes 42 are
19 preferably readily insertable and removable in and from bore 34 or 34A as the case may
20 be, hence may be considered to be "disposables". Particularly, the "disposable" shown
21 in Fig. 6 lends itself to laboratory preloading and packaging in hermetically sealed
22 containers from convenient distribution and use. In both the embodiments of Figs. 5 and
23 6, the materials forming both lens 32 and tube 42A are selected so that the respective
24 indices of refraction thereof are substantially matched. In order to provide the optimum
25 optical coupling between tube 42A and lens 32, a refractive index-matching fluid is
26 preferably disposed around the tube in the interspace between the tube and the interior
27 wall of bore 34A.

28 It should be understood that bead mass 40 of the embodiment of Fig. 6 can be
29 formed by, for example, the same technique used to create the bead mass of Fig. 5, i.e.
30 by flowing a suspension of beads through conduit 42 to accrete against a screen such as
31 38B, the other screen then being emplaced to capture the bead mass. Alternatively, the
32 porous bead mass may also be formed of a plurality of bead adhered lightly to one
33 another as by sintering or adhesives. For example, the bead mass can be formed by
34 providing a thick layer of beads which may be free-standing, or by coating a porous
35 substrate or forming a sandwich between a pair of porous substrates, with the thick layer

1 of beads, which bead layers include a minor amount of adhesive that will not materially
2 reduce the porosity of the resulting mass. After curing, the coating can be precoated with
3 an appropriate specifically reactive ligand and minute cylinder of the coating punched out
4 and inserted into appropriately dimensioned tubes 42. Alternatively, sheets of high-
5 molecular weight polymeric material of the desired porosity are commercially available,
6 and after treatment to immobilize the requisite ligand within the porous structure, can be
7 punched to produce the desired cylinders for insertion into tubes 42. Thus, one may
8 provide a plurality of bead masses, each coated with a different ligand. The resulting
9 plurality of bead masses can be emplaced in a single tube 42, as shown in Fig. 7, so that
10 one may assay a sample flowing through the tube for several different ligands separately
11 but substantially simultaneously.

12 As shown in Fig. 8, conduit 34 can be formed in part as a shallow elongated
13 channel 34B or hemi-tubular portion of, for example, semicircular cross-section cut or
14 molded into planar surface 48 of lens 33 which extends perpendicularly to the optical axis
15 of the lens and through the focal region of lens means 32. The remainder of conduit 34
16 is formed by another hemi-tubular elongated channel 34C, similarly of semicircular cross-
17 section, provided in plate 50. The latter is attached to lens 32 adjacent surface 48,
18 typically by hinging 52 such that plate 50 can be rotated to match channels 34C and 34B
19 into coaxial relation to form a combined conduit of substantially circular cross-section.
20 In the preferred embodiment, the inner surface of channel 34C is provided with highly
21 reflective coating 54.

22 Since certain changes may be made in the above process and apparatus without
23 departing from the scope of the invention herein involved, it is intended that all matter
24 contained in the above description or shown in the accompanying drawing shall be
25 interpreted in an illustrative and not in a limiting sense.

What is claimed is:

- 1 1. A flow cell for use in apparatus for assaying a fluid sample, said apparatus
2 comprising, in combination:
 - 3 hollow, light-transparent conduit means adapted for fluid flow therethrough; and
4 and
 - 5 a porous mass of light-transparent material disposed in said conduit means, the
6 porosity of said mass of transparent material being selected to permit fluid flow of said
7 sample therethrough, said mass having immobilized on the surfaces thereof, at least a
8 moiety of a ligand/conjugate complex.
- 1 2. A flow cell as defined in claim 1 including
2 fluid-porous barrier means disposed within said conduit means so as to define at
3 least one wall of a chamber in said conduit means; and wherein
4 said porous mass comprises a plurality of particles dimensioned within a specified
5 range of diameters, the porosity of said barrier means being sufficiently smaller than said
6 range so that said particles are trapped by said barrier means in said chamber to form said
7 porous mass.
- 1 3. Apparatus as defined in claim 2 wherein said barrier means comprises at least
2 a pair of screens spaced apart from one another so as to define a reaction chamber within
3 said conduit means, the mesh of said screens being sufficiently smaller than said range
4 of diameters so that said particles are trapped by said screens in said chamber to form
5 said porous mass, said particles having immobilized on the surfaces thereof, at least a
6 respective moiety of said ligand/conjugate complex.
- 1 4. Apparatus as defined in claim 2 wherein said barrier means comprises a
2 plurality of pairs of screens, the screen of each said pair being spaced apart from one
3 another so as to define a respective reaction chamber within said conduit means;
4 each said chamber including a plurality of particles dimensioned within a specified
5 range of diameters, the mesh of said screens being sufficiently smaller than said range
6 of diameters so that said particles are trapped by said screens in each said chamber to
7 form a respective porous mass, the particles of each said mass having immobilized on the
8 surfaces thereof, at least a respective moiety of a respective ligand/conjugate complex.

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1 5. A flow cell for use in apparatus for assaying a fluid sample, said apparatus
2 comprising, in combination:

3 focussing optical lens means;

4 a hollow, cylindrical passage through said lens means adapted for fluid flow
5 therethrough and extending transversely to the optical axis of said lens means through the
6 focal region of said lens means.

1 6. Apparatus as defined in claim 5 wherein said lens means comprises a plurality
2 of lenses and said passage comprises conduit means extending through one of said lenses.

1 7. Apparatus as defined in claim 6 including a porous mass of light-transparent
2 material disposed in said conduit means, the porosity of said mass of transparent material
3 being selected to permit flow of said fluid sample therethrough, said mass having
4 immobilized on the surfaces thereof, at least a moiety of a ligand/conjugate complex.

1 8. A flow cell as defined in claim 5 including mechanical, fluid-porous barrier
2 means dimensioned and disposed adjacent said focal region so as to block flow of
3 particulates of predetermined size through said conduit means.

1 9. Apparatus as defined in claim 6 including a first elongated hemi-tubular
2 channel in said one of said lenses and a second elongated hemi-tubular channel, said
3 channels being mateable with one another to define said conduit means.

1 10. Apparatus as defined in claim 9 wherein said second hemi-tubular channel
2 has a light reflective surface on the concave portion thereof.

1 11. Apparatus as defined in claim 9 wherein said one elongated edge of said first
2 channel is hingedly connected to an elongated edge of said second channel.

1 12. Apparatus as defined in claim 8 wherein said hollow conduit means
2 comprises a light-transparent tube positioned within said cylindrical passage, said barrier
3 means being disposed in said tube.

1 13. Apparatus as defined in claim 12 wherein said barrier means comprise a pair
2 of screens spaced apart from one another so as to define a reaction chamber within said

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3 tube; and
4 including a plurality of particles dimensioned within a specified range of
5 diameters, the mesh of said screens being sufficiently smaller than said range so that said
6 particles are trapped by said screens in said chamber to form a porous mass positionable
7 substantially at said focal region.

1 14. Apparatus as defined in claim 12 wherein the indices of refraction of said
2 lens means and said tube are substantially matched.

1 15. Apparatus as defined in claim 14 including an refractive index-matching fluid
2 disposed around said tube and in the interspace between said tube and said passage.

1 16. Apparatus as defined in claim 7 wherein one portion of said passage is
2 formed as an elongated hemi-tubular first channel in said lens means and the remaining
3 portion of said passage is formed as an elongated hemi-tubular second channel, matched
4 to said first channel.

1 17. Apparatus as defined in claim 16 wherein said second channel has a
2 reflective surface forming at least a part of the inner surface of said passage.

1 18. Apparatus as defined in claim 8 including a plurality of particles dimensioned
2 within a specified range of diameters, said fluid-porous means having pores of lesser
3 diameter than said range so that said particles are accreted in said conduit means against
4 said barrier means to form a porous mass disposed substantially at said focal region.

1 19. Apparatus as defined in claim 18 wherein said particles are substantially
2 transparent to light.
3

1 20. Apparatus as defined in claim 19 wherein said particles are coated on at least
2 on a portion of the surface thereof with at least a moiety of a ligand/conjugate complex.

1 21. Apparatus for assaying a fluid sample using a tag capable of emitting
2 electromagnetic radiation when excited, said apparatus comprising:
3 lens means capable of focussing said radiation and having conduit means therein
4 extending transversely of the optical axis of said lens means through the focal region of

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5 said lens means, said conduit means being adapted for fluid flow therethrough ;
6 fluid-porous barrier means disposed adjacent said focal region in said conduit
7 means for limiting the passage of particles through said porous means as a function of
8 particle size.

1 22. Apparatus as defined in claim 21 including means for exciting emission of
2 said electromagnetic radiation.

1 23. Apparatus as defined in claim 22 wherein said means for exciting said
2 emission comprise a source of excitation radiation and means for directing said excitation
3 radiation at said conduit means at said focal region.

1 24. Apparatus as defined in claim 23 wherein said emission is fluorescent
2 radiation.

1 25. Apparatus as defined in claim 23 including a plurality of particles
2 dimensioned within a specified range of diameters, said fluid-porous means having pores
3 of lesser diameter than said range so that said particles are accreted in said conduit means
4 against said porous barrier to form a porous mass disposed substantially at said focal
5 region.

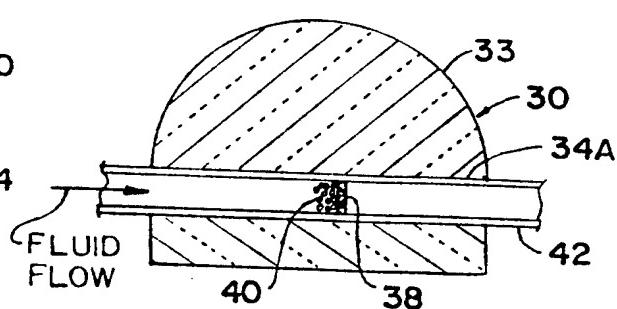
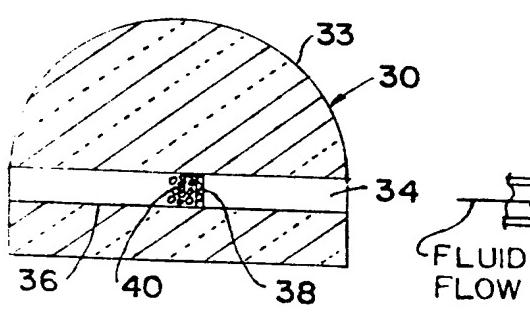
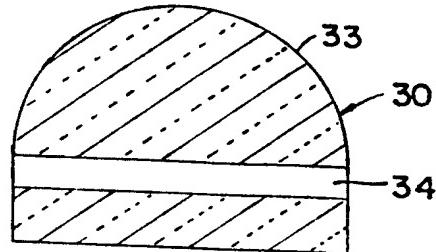
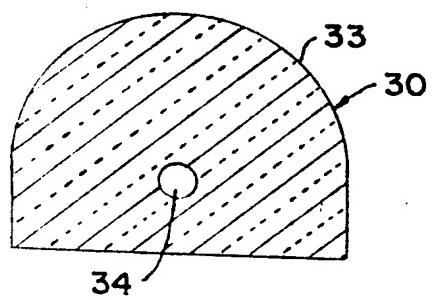
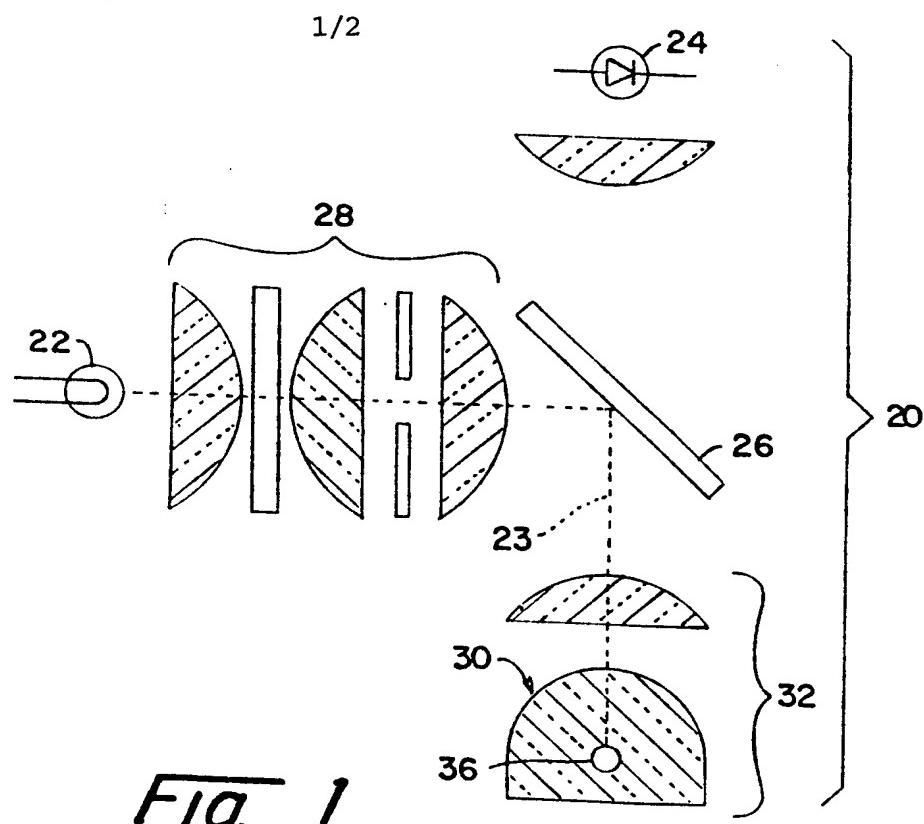
1 26. Apparatus as defined in claim 25 wherein said particles are substantially
2 transparent to both said excitation radiation and said fluorescent radiation, and are coated
3 with immobilized specific binding ligand.

1 27. Apparatus as defined in claim 26 wherein said ligand has formed a complex
2 in a ligand/conjugate reaction, said complex being tagged with molecules capable of
3 fluorescing when excited by appropriate excitation radiation.

1 28. Method of assaying a fluid sample by detection of radiation emitted from a
2 ligand/conjugate complex, said method comprising the steps of:
3 providing a plurality of particles dimensioned within a specified range of
4 diameters;
5 flowing a suspension of said particles through conduit means extending

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- 6 transversely of the optic axis of lens means through the focal region of the latter;
- 7 arresting the flow of said particles through said conduit by porous means having
- 8 pores of lesser diameter than said range, so that said particles accrete substantially at said
- 9 focal region;
- 10 treating said accretion of particles, including flowing at least said fluid sample
- 11 therethrough, so as to create said ligand/conjugate complex on the surfaces of said
- 12 particles;
- 13 stimulating said complex so that characteristic radiation arises therefrom; and
- 14 detecting emission of said characteristic radiation transmitted by said lens means.
- 1 29. Method of assaying as defined in claim 28 wherein said treating includes
- 2 coating said particles at least on a portion of the surface thereof with at least a moiety of
- 3 said complex;
- 4 tagging said complex with a fluorescent label; and
- 5 flowing through said conduit means and said accretion of said particles a wash
- 6 fluid to separate unreacted conjugate and fluorescent label from the tagged complex.
- 1 30. Method of assaying as defined in claim 28 wherein said stimulating includes
- 2 directing excitation radiation onto said tagged complex; and said characteristic radiation
- 3 comprises fluorescence arising from the excited tagged complex and passing through said
- 4 lens means.



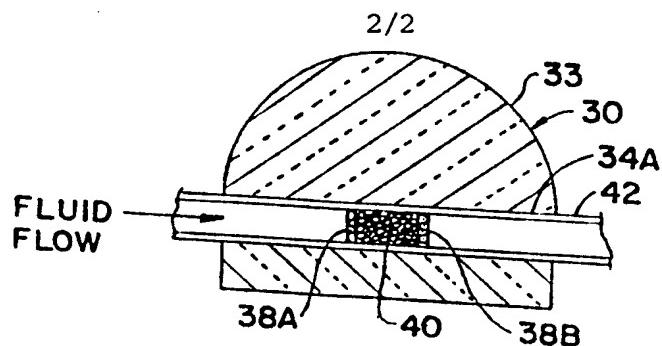


Fig. 6

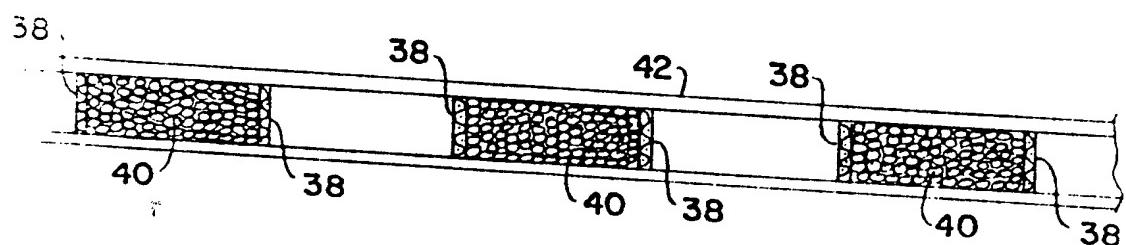


Fig. 7

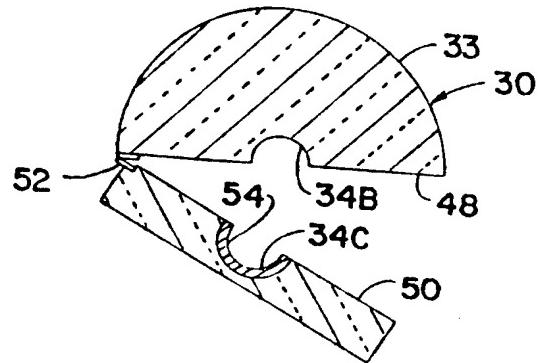


Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/06967

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61B 5/00;
US CL :422/82.07; 435/808

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

search terms: lens, assay, screen, filter, conduit, antigen, antibod?, optic?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US, A, 4,425,438 (BAUMAN et al) 10 January 1984, see entire document.	1,2 3,4,7,8,12-30
X Y	US, A, 4,714,345 (SCHRADER) 22 December 1987, see entire document.	5,6,9,10 4,8,11-30
Y	US, A, 3,025,142 (WILLIAMS) 13 March 1962, see entire document.	3,4,30
Y	US, A, 4,652,533 (JOLLEY) 24 March 1987, see entire document.	27,29,30
A	US, A, 4,059,685 (JOHNSON) 22 November 1977, see entire document.	1-30



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

31 August 1993

Date of mailing of the international search report

29 SEP 1993

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/06967

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 3,492,396 (DALTON et al) 27 January 1970, see entire document.	1-4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/06967

B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

422/82.07, 102, 68.01, 82.05, 82.08, 99, 104; 435/808; 436/ 164, 165, 172, 527, 538, 531, 546, 547, 805; 55/ 387,
485; 359/665; 356/246